LETTER

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T cells from patients with Parkinson's disease recognize α-synuclein peptides

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Genetic studies have shown the association of Parkinson's disease with alleles of the major histocompatibility complex[1–3]. Here we show that a defined set of peptides that are derived from α-synuclein, a protein aggregated in Parkinson's disease[4], act as antigenic epitopes displayed by these alleles and drive helper and cytotoxic T cell responses in patients with Parkinson's disease. These responses may explain the association of Parkinson's disease with specific major histocompatibility complex alleles.

Abnormal processing of self-proteins can produce epitopes, which are presented by major histocompatibility complex (MHC) proteins to be recognized by specific T cells that have escaped tolerance during thymic selection[5]. Such actions by the acquired immune system have been implicated in autoimmune disorders, including type-1 diabetes. While not considered to possess autoimmune features, neurodegenerative diseases are characterized by altered protein processing. The major pathological features of Parkinson's disease, the most common neurodegenerative movement disorder, are the death of dopaminergic neurons of the substantia nigra, and the presence of intraneuronal aggregates known as Lewy bodies that are composed of α-synuclein (α-syn)[4]. Activated microglia have been reported in the substantia nigra of patients with Parkinson's disease for nearly a century[6] and cytokine profiles have implicated the activation of the innate immune system[7]. More recent evidence has suggested a role for the acquired immune system[7], including T cell infiltration into the substantia nigra of patients with Parkinson's disease[8]. Genome-wide association studies have shown the association of Parkinson's disease with an immune haplotype[9] that is present in approximately 15% of the general population including the MHC class II gene alleles DRB5*01 and DRB1*15:01 (ref. 1), and a polymorphism in a non-coding region that may increase MHC class II expression[2,3]. We have reported antigen presentation by MHC class I expression in dopamine neurons of the substantia nigra in adult human brains of patients with Parkinson's disease and age-matched controls. We have further demonstrated that dopamine neurons of the substantia nigra express MHC class I upon activation by cytokines that are released from microglia, which are activated by α-syn or neuromelanin, and that $CD8^+$ T cells kill neurons that present the appropriate combination of MHC class I and peptide[10]. Native[11,12] and modified (nitrated) synuclein-derived peptides[13] elicit T cell responses in rats and mice, and it was previously demonstrated that neuronal death in the substantia nigra in an α-syn overexpression model is absent in MHC II null mice[14].

To address whether Parkinson's disease is associated with T cell recognition of epitopes that are derived from α-syn presented by specific MHC alleles, we recruited 67 participants with Parkinson's disease and 36 age-matched non-Parkinson's disease healthy controls. Participants were 46–83 years of age (Parkinson's disease, median 66, range 46–83; healthy controls, median 64, range 52–83) and 66% were male (Parkinson's disease, 75%; healthy controls, 50%) (Supplementary Tables 1, 2). Whereas approximately 15% of healthy controls carried DRB1*15:01 or DRB5*01:01 alleles, around one-third of patients with Parkinson's disease carried these alleles (difference between patients with Parkinson's disease and healthy controls, $P = 0.036$ and 0.022 for DRB1*15:01 and DRB5*01:01, respectively), indicating association of HLA DR allelic variants with Parkinson's disease in our cohort (Supplementary Table 3).

To determine whether α-syn-derived peptides were recognized by T cells, we assayed responses to pools that each contained approximately twenty peptides of 9–10 amino acids (a.a.) predicted to bind common HLA class I types[15], and peptides of 15 amino acids spanning the protein that could elicit HLA class II responses. Peripheral blood mononuclear cells (PBMCs) from patients with Parkinson's disease and healthy controls were stimulated for 14 days. Interferon-γ (IFNγ) and interleukin-5 (IL-5) responses were measured by dual-colour enzyme-linked immuospot (ELISPOT) assay, enabling quantification of responsive cells. Positive pools were deconvoluted to identify the peptides eliciting cytokine responses. IFNγ was used as a representative cytokine to detect $CD8^+$ HLA class I and $CD4^+$ T helper 1 (T_h1) class II T cells, and IL-5 as a representative cytokine secreted by $CD4^+$ T_h2 class II T cells. Each pool was tested in an initial cohort of 19–25 randomly selected patients with Parkinson's disease and 12 healthy controls. The majority of PBMC responses to the peptides of 15 amino acids produced IL-5 (68% of total responses), indicating a prominent $CD4^+$ T_h2 phenotype, and the remainder of the responses were to IFNγ (32%). No cells producing both IL-5 and IFNγ were detected.

We identified two antigenic regions in α-syn, the first near the N terminus, composed of a.a.31GKTKEGVLYVGSKTKa.a.45 and a.a.32KTKEGVLYVGSKTKEa.a.46 (referred to as the Y39 region) (Fig. 1a), which elicited an apparent class II restricted IL-5 and IFNγ response (Fig. 1b–d). Residue 32 is a plasmin-cleavage site[16] and chymotrypsin-cleavage digestion sites are at 32 and 45 (ref. 17).

The second antigenic region was near the C terminus (a.a. 116–140) (referred to as the S129 region) (Fig. 1a) and required phosphorylation of amino acid residue S129. The three phosphorylated S129 epitopes (a.a.116MPVDPDNEAYEMPSEa.a.130, a.a.121DNEAYEMPSEEGYQDa.a.135, a.a.126EMPSEEGYQDYEPEAa.a.140) produced markedly higher IL-5 responses in patients with Parkinson's disease than in

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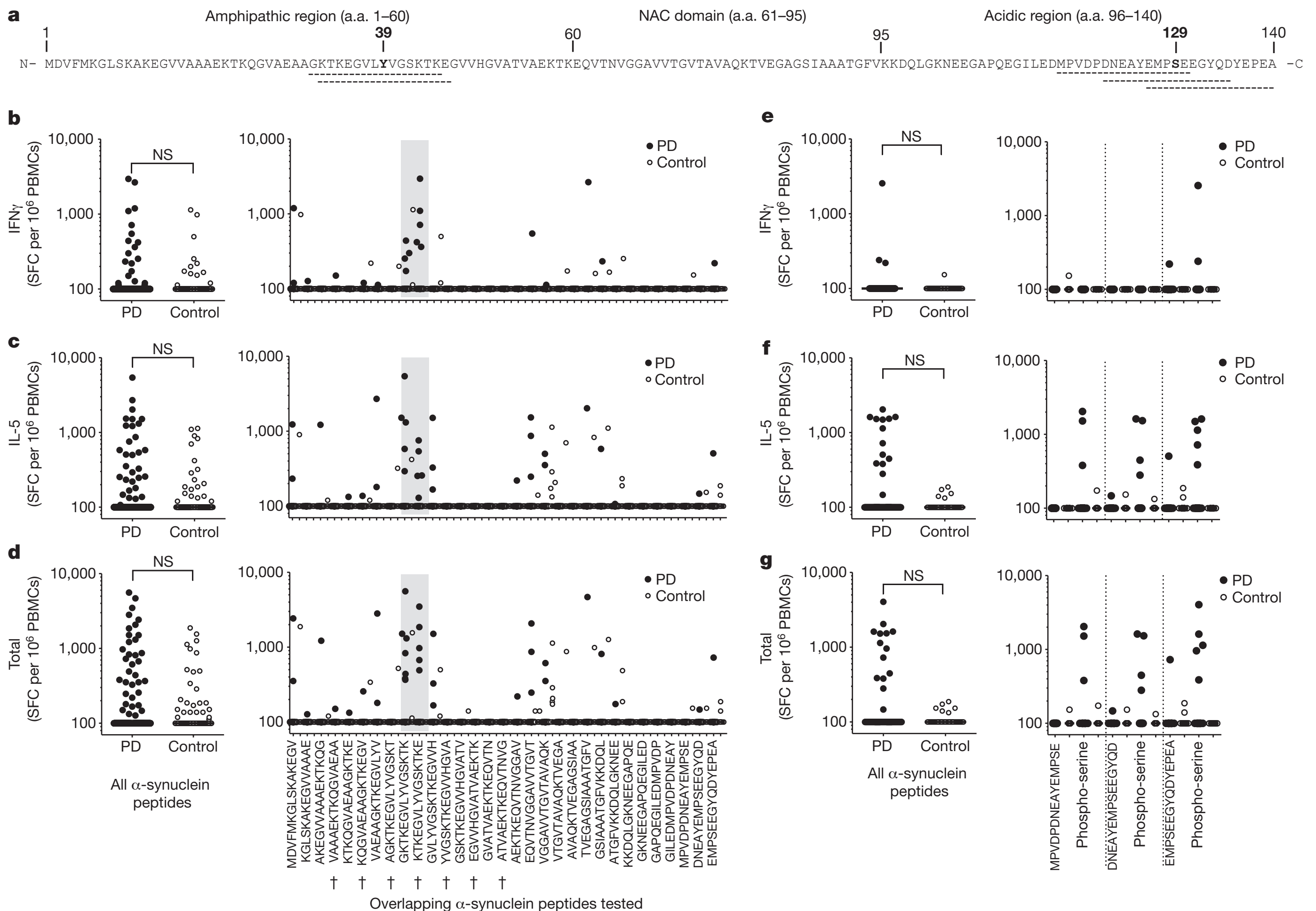

Figure 1 | α-syn autoimmune responses are directed against two regions. a, Sequence of α-syn. Antigenic regions are highlighted with dashed lines with amino acids Y39 and S129 shown in bold. **b–d**, Magnitude of responses expressed as SFC per 10^6 PBMCs per peptide and participant combination. Left, response to all overlapping native α-syn 15-mer peptides in patients with Parkinson's disease (PD, $n = 733$) and control ($n = 372$). Right, responses against specific 15-mers. Grey shading indicates the antigenic region containing Y39. **e–g**, Magnitude of responses. Left, responses to all native and phosphorylated S129 α-syn 15-mer peptides in patients with Parkinson's disease ($n = 150$) and control ($n = 72$). Right, responses against specific S129 peptides. Closed circles, patients with Parkinson's disease ($n = 19$, peptides indicated by a dagger (†); $n = 25$, all other peptides); open circles, control ($n = 12$ participants). Two-tailed Mann–Whitney *U*-test; NS, not significant. **b, e**, IFNγ response. **c, f**, IL-5 response. **d, g**, Total (combined IFNγ and IL-5) response. Because many participants showed no response, many points are at the limit of detection (100 SFC).

healthy controls ($P = 0.02$, Fisher's exact test, threshold of at least 300 spot-forming cells (SFC)) (Fig. 1e–g). Phosphorylated S129 residues are present at high levels in Lewy bodies of patients with Parkinson's disease[18], and Lewy bodies of patients with Parkinson's disease contain α-syn fragments with cleavage sites approximately at amino acids 115, 119, 133 and 135 (ref. 19), and include the fragment a.a.129SEEGYQDYEPEAa.a.140, which is contained within one of the S129 epitopes. Caspase-1 (ref. 20) and neurosyn[21] can cleave α-syn at a.a.121, chymotrypsin and cathepsin D digestion sites are at a.a.116, a.a.125 and a.a.136 (ref. 17), proteasome may cleave between a.a.119 and a.a.120 (ref. 22), and calpain can cleave at a.a.122, with resulting fragments that have been identified in brains of patients with Parkinson's disease[23].

The immune responses to a.a.39 and a.a.129 region epitopes, which included analysis of a second cohort of 19 patients with Parkinson's disease and 12 healthy controls that were assayed for response to additional phosphorylated and nitrated modifications (Extended Data Fig. 1), were different between patients with Parkinson's disease and healthy controls for secretion of IFNγ (two-tailed Mann–Whitney *U*-test, $P < 0.05$), IL-5 (two-tailed Mann–Whitney *U*-test, $P < 0.001$) and both combined responses (two-tailed Mann–Whitney *U*-test, $P < 0.001$) (Fig. 2a–c). While residue 39 is highly phosphorylated in patients with Parkinson's disease[24], Y39 phosphorylation was not required for antigenic response. The response was primarily polarized towards IL-5 in patients with Parkinson's disease (71% IL-5 and 29% IFNγ; Fig. 2d). This polarization was specific to patients with Parkinson's disease, and the relatively rare responses in healthy controls were not similarly polarized (46% IL-5 and 54% IFNγ).

To identify specific sets of T cells that respond to α-syn epitopes, we measured response to a pool of the 11 α-syn antigenic peptides by nine participants with Parkinson's disease (Extended Data Fig. 2). Approximately 0.2% of $CD3^+$ T cells responded to the α-syn peptides. Of the responsive T cells, approximately 50% produced IL-4 and 50% produced IFNγ, with no detectable IL-10 or IL-17 production. In most cases, responses were mediated by $CD4^+$ T cells, but response by one patient with Parkinson's disease was mostly mediated by IFNγ-producing $CD8^+$ T cells. Therefore, the T cell response to α-syn antigenic peptides was largely mediated by IL-4 or IFNγ-producing $CD4^+$ T cells, with potential contributions from IFNγ-producing $CD8^+$ T cells.

To test whether the α-syn epitopes arise from processing of native and/or fibrilized α-syn, PBMCs were stimulated with α-syn epitopes for 14 days. The cultures were then assayed following exposure to α-syn peptides, 25 μg ml^{-1} fibrilized (pre-formed fibrils, PFF) α-syn,

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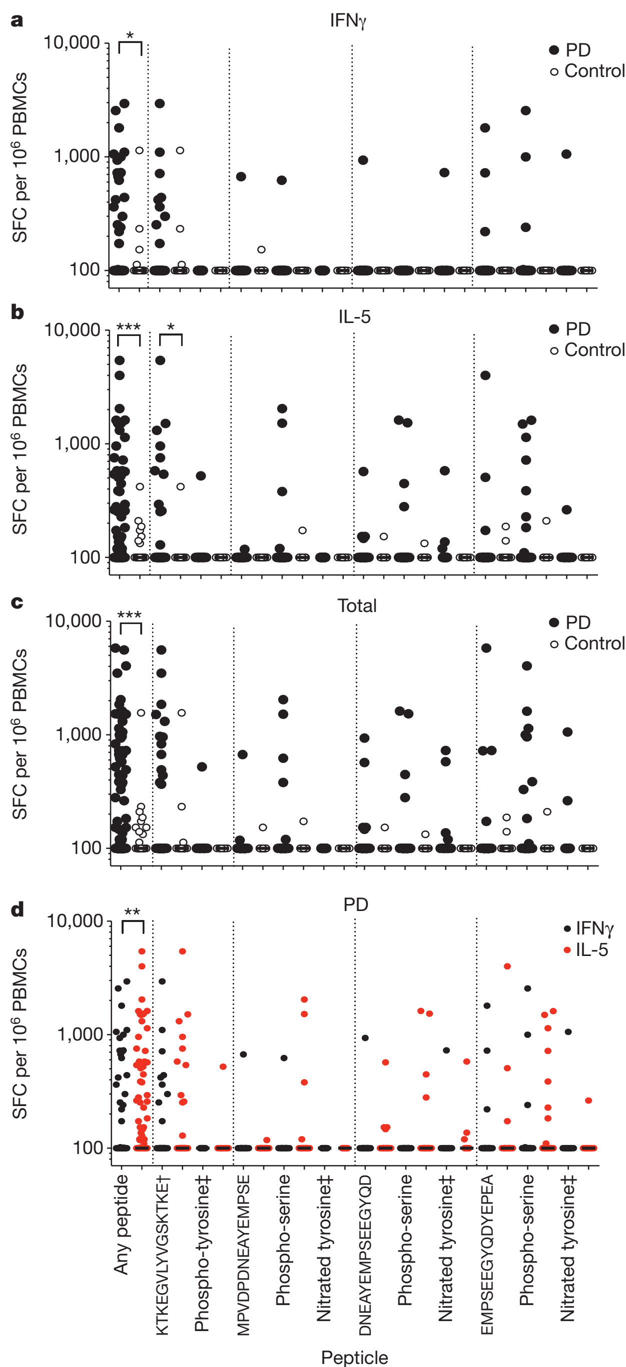

Figure 2 | Reactivity to native and modified α-syn peptides in patients with Parkinson's disease. a–c, Magnitude of responses against native and modified α-syn 15-mer S129 and Y39 region peptides as SFC per 10^6 PBMCs. Each point represents a peptide and participant combination. Closed circles, patients with Parkinson's disease ($n=403$ peptide and participant combinations (any peptide); KTKEGVLYVGSKTKE $n=63$ participants (†); the modified peptides marked with a double dagger (‡) are tested in 19 participants; unmodified peptides are tested in $n=41$); open circles, control ($n=228$ any peptide; †$n=36$; ‡$n=12$; and unmodified peptides $n=24$). **a**, IFNγ response. **b**, IL-5 response. **c**, Total (combined IFNγ and IL-5) response. **d**, Combined IL-5 and IFNγ responses against individual native and modified α-syn peptides by patients with Parkinson's disease. Black points, IFNγ responses; red points, IL-5 responses. Two-tailed Mann–Whitney *U*-test; $*P<0.05$, $**P<0.01$, $***P<0.001$. As many participants showed no response, many points are at the limit of detection (100 SFC).

25 μg ml^{-1} native α-syn or medium alone. Extended Data Figure 3 shows that T cell lines specific for the α-syn epitopes were activated by antigen-presenting cells pulsed with native or PFF protein in 7 out of 12 or 11 out of 12 cases, respectively. There was a significantly higher response to native α-syn ($P=0.004$) and to PFF α-syn ($P=0.0005$) than to medium alone. Therefore, T cells can respond to α-syn epitopes arising from natural processing of extracellular native α-syn, which is present in blood, and the fibrilized α-syn associated with Parkinson's disease.

We then identified the HLA alleles that present α-syn peptides by *in vitro* binding to a panel of HLAs representing the common alleles expressed in worldwide populations[1]. A threshold of 1,000 nM binding affinity is associated with immunogenicity of HLA class II T cell epitopes, and most epitopes bind in the 1–100 nm range, with affinities in the 1–10 nM considered to be of high affinity. Of 26 common HLA class II alleles tested, five bound to a.a.32KTKEGVLYVGSKTKEa.a.46 (Supplementary Table 4). The HLA class II variants DRB1*15:01 and DRB5*01:01 bound to the epitope with high affinity (2.8 nM and 8.1 nM, respectively), while DRB1*07:01, DRB1*09:01 and DQB1*03:01 bound in the 80–250 nM range. The a.a.32KTKEGVLYVGSKTKEa.a.46 epitope phosphorylated at Y39 also bound DRB1*15:01 and DRB5*01:01 with high affinity. Comparison of patients with Parkinson's disease with and without DRB1*15:01 alleles showed that there was no difference in levels of HLA class I or class II protein expression (Extended Data Figs 4, 5). Thus, epitopes in the Y39 region of α-syn strongly bind HLA heterodimers including two HLA class II β chain alleles associated with Parkinson's disease.

By contrast, the C terminus peptides spanning S129 and its post-translational forms bound HLA class II alleles weakly, with the exception of a.a.121DNEAYEMPSEEGYQDa.a.135, which in both native and phosphorylated S129 forms strongly bound to DQB1*05:01. The a.a.116MPVDPDNEAYEMPSEa.a.130 epitope bound to several alleles with lower affinity, and the a.a.126EMPSEEGYQDYEPEAa.a.140 epitope bound to DQB1*04:02 and DQB1*05:01 with low affinity. Thus, antigenic peptides in the C terminus S129 antigenic region demonstrated relatively little clear restriction, suggesting that they are recognized promiscuously.

DRB1*15:01 and DRB5*01:01 alleles are in linkage disequilibrium, and participants expressing one allele are likely to express both. Of participants with Parkinson's disease, 8 out of 13 responders to the a.a.32KTKEGVLYVGSKTKEa.a.46 epitope expressed both DRB1*15:01 and DRB5*01:01, while only 12 out of 45 (DRB1*15:01) and 13 out of 43 (DRB5*01:01) non-responders expressed the alleles, indicating an association between the alleles and antigenic response (odds ratios of 4.4 and 3.7, *P* values of 0.04 and 0.05, respectively)

Table 1 | HLA association of Y39 responses

	HLA allele	Individuals with allele		Individuals lacking allele		Relative frequency	Odds ratio	*P* value
		Positive epitope response	Negeagative epitope response	Positive epitope response	Negative epitope response			
PD	DRB1*15:01	8	12	5	33	1.8	4.4	0.04
PD	DQB1*03:04	2	0	11	45	0	11	0.05
PD	DRB5*01:01	8	13	5	30	1.6	3.7	0.05
PD	DRB3*02:02	1	19	12	24	0.2	0.1	0.021
PD	A*11:01	8	9	5	36	2.1	6.4	0.012
PD	DRB1*15:01/ DQB1* 03:04/DRB5* 01:01/A*11:01	13	18	0	27	1.9	inf.	0.00007
HC	DRB1*15:01/ DQB1* 03:04/DRB5* 01:01/A*11:01	3	5	0	26	4.3	inf.	0.009

HC, healthy control participants; PD, participants with Parkinson's disease. Inf, infinite.

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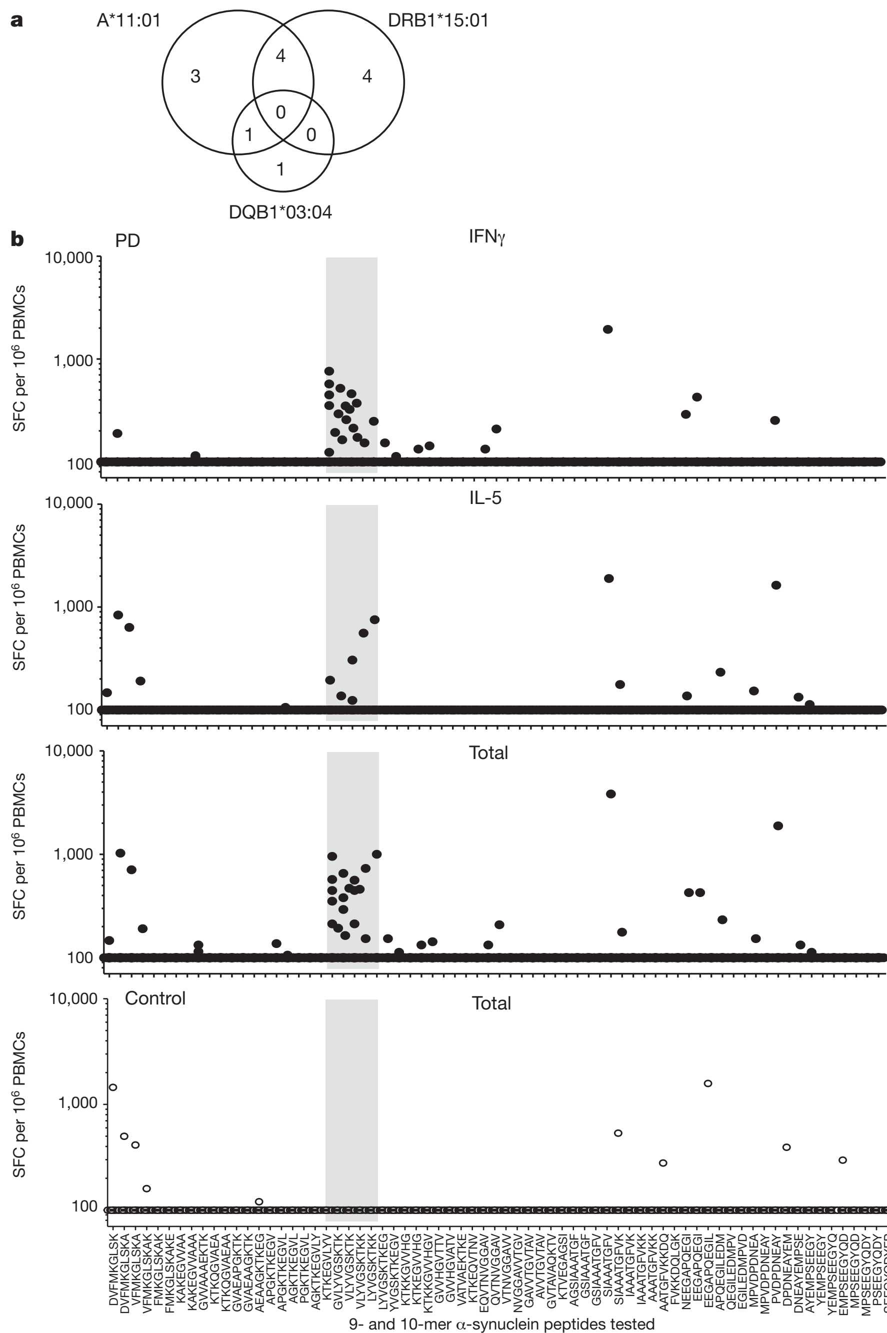

Figure 3 | HLA association of the Y39 epitope and identification of A*11:01 restricted 9–10 a.a. length Y39 epitopes. a, Overlapping but largely independent associations between DRB1*15:01, DQB1*03:04 and A*11:01 for Parkinson's disease (13 participants) responding to the Y39 epitope. **b**, **c**, Magnitude of responses by patients with Parkinson's disease (**b**, $n = 19$) and control participants (**c**, $n = 12$), as SFC per 10^6 PBMCs of response per peptide and participant combination to α-syn 9–10-mer peptides spanning the protein. **b**, Top, IFNγ; middle, IL-5; bottom, total (combined IFNγ and IL-5) response in patients with Parkinson's disease. **c**, Total (combined IFNγ and IL-5) response in control participants. As many participants showed no T cell response, many points are at the limit of detection (100 SFC).

(Table 1). This analysis detected additional associations, with 2 out of 13 responders expressing DQB1*03:04 ($P = 0.05$) compared to 0 out of 45 non-responders, as well as the HLA class I allele A*11:01, with 8 out of 13 responders expressing A*11:01 compared to 9 out of 45 non-responders ($P = 0.012$). While A*11:01 is in relatively mild linkage disequilibrium with DRB1*15:01 and DRB1*01:01, the associations were largely independent (Fig. 3a). In general, participants with Parkinson's disease showed a trend towards higher expression of HLA molecules, particularly HLA class II. This is consistent with an inflammatory component of Parkinson's disease, and higher HLA class II expression and induction in PBMCs of patients with Parkinson's disease compared to healthy controls[3]. Little or no difference in HLA class II expression was found between participants expressing DRB1*15:01 versus other DRB1 alleles (Extended Data Fig. 4). A similar but still less pronounced trend was noted for HLA class I (Extended Data Fig. 5). This suggests that the association between DRB1*15:01 and Parkinson's disease is not based on differential expression of the protein. We detected a negative association between recognition of a.a.32KTKEGVLYVGSKTKEa.a.46 and the DRB3*02:02 allele, suggesting this allele might be protective. The four alleles DRB1*15:01, DRB5*01:01, DQB1*03:04 and A*11:01 accounted for every single individual responding to the a.a.39 epitope ($P = 0.00007$ for Parkinson's disease, Table 1). This association was far more significant in Parkinson's disease than healthy controls ($P = 0.009$). The combined association

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of the four alleles for Parkinson's disease versus healthy controls was significant ($P=0.008$ two-tailed Fisher's exact test compared to individual DRB1*15:01, $P=0.05$; and DRB5*01:01, $P=0.03$), with around half of the patients with Parkinson's disease (31 with alleles and 27 without) carrying one of the four alleles, whereas only around 20% of the healthy controls (8 with alleles and 26 without) expressed one of the four (Table 1).

Following detection of association of response to the Y39 region with the MHC class I allele HLA A*11:01, we evaluated Parkinson's disease responses to shorter α-syn-derived peptide candidates for class I presentation. We found that 5 out of 19 patients with Parkinson's disease responded to these short peptides, whereas 0 out of 12 healthy controls responded (Fig. 3b, c) (two-tailed $\chi_1^2=3.765, P=0.0523$). Reactivity occurred mostly on peptides contained within the Y39 region, involving three peptides (a.a.36GVLYVGSKTKa.a.45, a.a.37VLYVGSKTKa.a.45, a.a.37VLYVGSKTKKa.a.46) predicted as potential A*11:01 binders[15]. We tested each peptide for binding to purified HLA A*11:01 molecules *in vitro*, and found that the 9-mer a.a.37VLYVGSKTKa.a.45, which is nested within the two 10-mers, bound with good affinity (50% inhibitory concentration (IC_{50}) = 161 nM), while the other two bound poorly, indicating that the 9-mer is responsible for T cell recognition. Reactivity to short peptides was mostly mediated by IFNγ-producing cells and most pronounced for the A11 binding peptides. Therefore, immune responses to α-syn associated with Parkinson's disease have both MHC class I and II restricted components.

Alleles of over twenty genes are associated with familial Parkinson's disease[25], many of which encode proteins implicated in lysosomal degradation pathways including mitochondrial turnover. For example, mutations in α-syn or dopamine-modified α-syn[26,27], and LRRK2 (ref. 28) interfere with protein degradation by chaperone-mediated autophagy, a process that becomes less efficient with age. Extracellular oligomeric α-syn may be acquired by brain cells during Parkinson's disease pathogenesis[29]. These reports suggest that altered degradation of proteins including α-syn could produce antigenic epitopes that trigger immune reactions during ageing and Parkinson's disease.

Our results indicate that peptides derived from two regions of α-syn produce immune responses in patients with Parkinson's disease; their roles in additional synucleinopathies are untested. Epitopes derived from the Y39 region (aproximately from a.a.31/32 to a.a.45/46) are specifically displayed by two MHC class II β-chain alleles, DRB5*01:01 and DRB1*15:01, associated with Parkinson's disease, as well as an additional MHC class II allele and an MHC class I allele not previously associated with Parkinson's disease. This response is enacted mostly by IL-5-secreting $CD4^+$ T cells, as well as IFNγ-secreting $CD8^+$ cytotoxic T cells. α-syn is, to our knowledge, not endogenously expressed by cells that express MHC class II, but is found in cerebrospinal fluid[30], from where it can be acquired by MHC class II-expressing cells. This situation is analogous to the experimental autoimmune encephalitis model of multiple sclerosis, as myelin proteins used to produce autoimmunity are not endogenous to MHC class II-expressing cells, but are accumulated and processed for MHC class II display by antigen-presenting cells and microglia. The Y39 antigenic region is strikingly close to the α-syn mutations that cause Parkinson's disease (A30P, E46K, H50Q, G51D, A53T)[25]. The second antigenic region encompasses S129 and requires S129 phosphorylation, a form present in Lewy bodies[18]; antigenic epitopes from that region are not strongly restricted and can drive immune responses in patients who do not express HLA alleles that recognize the Y39 region.

Approximately 40% of the participants with Parkinson's disease in our cohort exhibited immune responses to α-syn epitopes, and these responses may reflect variations in disease progression or environmental factors. The fraction of patients who display these responses in classic autoimmune disorders such as type-1 diabetes, rheumatoid arthritis and multiple sclerosis is often around 20–50%[31,32]. As with type-1 diabetes, which features epitopes that are derived from both preproinsulin and additional proteins, it may be that epitopes related to Parkinson's disease are derived from α-syn and additional proteins. In classic autoimmune disorders, the MHC class II response may precede MHC class I (ref. 5), and we note that exposing microglia to α-syn triggers MHC class I expression by dopamine neurons[10]. The Parkinson's disease-associated proteins parkin and PINK1 may regulate antigenic presentation of mitochondrial peptides[33], and it is possible that an autoimmune presentation of antigenic epitopes unites lysosomal and mitochondrial mechanisms of Parkinson's disease pathogenesis.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions D.S. and A.S. conceived the study and wrote the paper. C.S.L.A. and F.G. contributed to writing and prepared figures. R.N.A. and L.C. recruited participants and performed clinical evaluations. C.L., J.A.-L. and A.F. maintained patient data and assisted in subject recruitment. E.K. arranged tissue handling and maintained records. F.G., C.O., J.P., M.B.D., C.C., D.W., E.P., S.M., B.P., W.H.H., C.M. and C.S.L.A. conducted analyses of T cells and antigenic epitopes. X.M., V.L.D. and T.M.D. prepared and characterized α-syn proteins and fibrils. J.S. performed *in vitro* MHC-binding assays.

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METHODS

Study subjects. All participants provided written informed consent for participation in the study. Ethical approval was obtained from the LJI and Columbia University.

We recruited 67 participants with Parkinson's disease and 36 age-matched healthy controls from the greater San Diego (Parkinson's disease, $n=9$; healthy controls, $n=13$) and New York City (Parkinson's disease, $n=58$; healthy controls, $n=23$) areas. The New York cohort was recruited from the Center for Parkinson's Disease at Columbia University Medical Center through the Spot study[34]. Blood samples were collected by S. Campbell and S. Yang of the Columbia Center for Translational Immunology (CCTI) Human Studies Core and approved by the CUMC Institutional Review Board. Parkinson's disease was defined based on the UK Parkinson's Disease Brain Bank criteria, without excluding cases with a family history of Parkinson's disease[35]. We collected demographics and disease characteristics including age, age of onset, sex, medications, comorbidities and motor disease severity as measured by the Unified Parkinson's Disease Rating Scale (UPDRS) motor score (UPDRS-III). We also collected family history of Parkinson's disease in first-degree relatives. The data are reported in Supplementary Table 1a, b. In the San Diego cohort, we collected demographic data and Parkinson's disease was self-reported. Samples used for additional assays in Fig. 3 and Extended Data Fig. 3 were collected from consecutive individuals based on the schedule of their appointment; the demographics and Parkinson's disease characteristics of these participants are shown in Supplementary Tables 2, 3. Healthy controls were recruited through a convenience sample of consecutive non-blood related individuals, and were mostly spouses of participants with Parkinson's disease. At Columbia University, Parkinson's disease and healthy controls were recruited only if there was no history of immune modulatory medications (for example, steroids) or overt autoimmune disorder (for example, lupus). No significant difference was detected in response rates as a function of sex or geographical location. Three participants with Parkinson's disease had a history of Crohn's disease and one patient had a history of Hashimoto's thyroiditis. Two of the three participants with Crohn's disease showed antigenic response to α-syn and the participant with Hashimoto's thyroiditis did not. Experimental blinding was accomplished by labelling the blood samples in a coded fashion without information on age/gender or Parkinson's disease status. The cohort was predominantly Caucasian (88.3%) and no firm conclusions between Crohn's disease and Parkinson's disease could be drawn because of the limited number of Crohn's disease patients studied.

Peptides. Peptides were synthesized as crude material on a small (1 mg) scale by A and A, LLC (San Diego). Peptides were forty 15-mers overlapping by 10–14 residues and seventy 9- or 10-mers predicted to bind common HLA class I alleles. In brief, each possible 9- and 10-mer from α-syn was scored for their capacity to bind a panel of 27 common HLA class I A and B molecules[36]. For each allele four peptides were synthesized (two 9-mers and two 10-mers, $n=61$ after removing redundant sequences that were selected for 2 or more alleles). In addition, any peptide that scored at the 2 percentile level or better for predicted binding, but were not within the four selected per allele were synthesized ($n=9$). Post-translationally modified peptides ($n=7$) were synthesized as purified material (>95% by reversed phase HPLC) by A and A, LLC (San Diego). Peptides were combined into pools of 14 peptides (range 11–16).

An alternative mode of stimulation would be to use whole α-syn protein, but we opted for synthetic peptides owing to their well-characterized and uniform chemical species, in contrast to α-syn preparations that contain varying amounts of different post-translational modifications, and as it is unclear which form(s) are processed by antigen-presenting cells during Parkinson's disease. In addition to a lower cost, synthetic peptides provide better mapping of specific epitopes and measurement of HLA binding.

PBMC isolation and culture. Venous blood was collected in heparin-containing blood bags or tubes. PBMCs were purified from whole blood by density-gradient centrifugation, according to the manufacturer's instructions. Cells were cryopreserved in liquid nitrogen suspended in FBS containing 10% (vol/vol) DMSO. Culturing of PBMCs for *in vitro* expansion was performed by incubating in RPMI (Omega Scientific) supplemented with 5% human AB serum (Gemini Bioscience), GlutaMAX (Gibco), and penicillin and streptomycin (Omega Scientific) at 2×10^6 per ml in the presence of individual peptide pools at 5 μg ml^{-1}. Every three days, 10 U ml^{-1} IL-2 in medium was added to the cultures.

ELISPOT assays. After 14 days of culture with individual peptide pools (5 μg ml^{-1}), the response to pools and individual peptides (5 μg ml^{-1}) was measured by IFNγ and IL-5 dual ELISPOT[37]. ELISPOT antibodies, mouse anti-human IFNγ (clone 1-D1K), mouse anti-human IL-5 (clone TRFK5), mouse anti-human IFNγ–HRP (clone 7-B6-1), mouse anti-human IL-5 biotinylated (clone 5A10) were all from Mabtech. To be considered positive, a response had to match three criteria: (1) elicit at least 100 spot-forming cells (SFC) per 10^6 PBMC; (2) $P \leq 0.05$ by Student's *t*-test or by a Poisson distribution test; (3) stimulation index ≥ 2.

For the experiments with fibrilized or native α-syn, PBMCs were stimulated with epitopes derived from α-syn for 14 days. These cultures were then stimulated with α-syn peptides, 25 μg ml^{-1} fibrilized α-syn or 25 μg ml^{-1} native α-syn.

HLA typing, restriction, binding predictions and assays. Participants were HLA-typed at the La Jolla Institute or by an American Society for Histocompatibility and Immunogenetics (ASHI)-accredited laboratory at Murdoch University (Western Australia). Typing at LJI was performed by next-generation sequencing[38]. Specifically, amplicons were generated from the appropriate class II locus for exons 2 through 4 by PCR amplification. From these amplicons, sequencing libraries were generated (Illumina Nextera XT) and sequenced with MiSeq Reagent Kit v3 as per the manufacturer's instructions (Illumina). Sequence reads were matched to HLA alleles and participant genotypes were assigned. HLA typing in Australia for class I (HLA A; B; C) and class II (DQA1; DQB1, DRB1 3,4,5; DPB1) was performed using locus-specific PCR amplification on genomic DNA. Primers used for amplification employed patient-specific barcoded primers. Amplified products were quantified and pooled by subject and up to 48 subjects were pooled. An unindexed (454 eight-lane runs) or indexed (8 indexed MiSeq runs) library was then quantified using Kappa universal QPCR library quantification kits. Sequencing was performed using either a Roche 454 FLX+ sequencer with titanium chemistry or an Illumina MiSeq using 2 × 300 paired-end chemistry. Reads were quality-filtered and passed through a proprietary allele-calling algorithm and analysis pipeline using the latest IMGT HLA allele database as a reference. The algorithm was developed by E.P. and S.M. and relies on periodically updated versions of the freely available international immunogenetics information system (http://www.imgt.org) and an ASHI-accredited HLA allele caller software pipeline, IIID HLA Analysis Suite (http://www.iiid.com.au/laboratory-testing/).

Potential HLA-epitope restrictions were inferred using the RATE program[39]. HLA A*11:01 binding predictions were performed using the consensus prediction method publicly available through the Immune Epitope Database (IEDB) Analysis Resource (available at http://www.iedb.org)[15].

Classical competition assays to quantitatively measure peptide-binding affinities for HLA class I and II MHC molecules, based on inhibition of binding of high affinity radiolabelled peptides to purified MHC molecules, were performed as detailed elsewhere[40]. In brief, 0.1–1 nM of radiolabelled peptide was co-incubated at room temperature or 37 °C with purified MHC in the presence of a cocktail of protease inhibitors (and, for class I, exogenous human β2-microglobulin). Following a two to four day incubation, MHC-bound radioactivity (c.p.m.) was determined by capturing MHC–peptide complexes on Lumitrac 600 plates (Greiner Bio-one, Frickenhausen, Germany) coated with either HLA DR (L243), DQ (HB180), DP (B7/21) or class I (W6/32) specific monoclonal antibodies. Bound c.p.m. was measured using the TopCount microscintillation counter (Packard Instrument Co.). The concentration of peptide yielding 50% inhibition of binding of the radiolabelled peptide was calculated. Under the conditions used, where [label] < [MHC] and $IC_{50} \geq$ [MHC], measured IC_{50} values are reasonable approximations of true K_d[41,42]. Each competitor peptide was tested at six different concentrations covering a 100,000-fold range, and in three or more independent experiments. As a positive control, the unlabelled version of the radiolabelled probe was also tested in each experiment. A threshold of 1,000 nM binding affinity is associated with immunogenicity of HLA class II T cell epitopes, and most epitopes bind in the 1–100 nm range, with affinities in the 1–10 nM considered to be of high affinity[43].

Intracellular cytokine staining. After 14 days of culture, PBMCs were stimulated in the presence of 5 μg ml^{-1} α-syn peptide pool for 2 h in complete RPMI medium at 37 °C with 5% CO_2. After 2 h, 2.5 μg ml^{-1} each of BFA and monensin was added for an additional 4 h at 37 °C. Unstimulated PBMCs were used to assess nonspecific/background cytokine production and PHA stimulation at 5 μg ml^{-1} was used as a positive control. After a total of 6 h, cells were collected and stained for cell surface antigens CD4 (anti-CD4–APCeF780, RPA-T4, eBioscience), CD3 (anti-CD3–AF700, UCHT1, BD Pharmingen), CD8 (anti-CD8–BV650, RPA-T8, BioLegend), CD14 (anti-CD14–V500, M5E2, BD Pharmingen), CD19 (anti-CD19–V500, HIB19, BD Pharmingen), and fixable viability dye eFluor 506 (eBioscience). After washing, cells were fixed using 4% paraformaldehyde and permeabilized using saponin buffer. Cells were stained for IFNγ (anti-IFNγ–APC, 4S.B3, eBioscience), IL-17 (anti-IL-17-PECy7, eBio64DEC17, eBioscience), IL-4 (anti-IL-4–PE/Dazzle594, MP4-25D2, BioLegend), and IL-10 (anti-IL-10-AF488, JES3-9D7, eBioscience) in saponin buffer containing 10% FBS. Samples were acquired on a BD LSR II flow cytometer. Frequencies of $CD3^+$ T cells responding to α-syn peptide pool were quantified by determining the total number of gated $CD3^+$ and cytokine$^+$ cells and background values were subtracted (as determined from the medium alone control) using FlowJo X Software (FlowJo). Combinations of cytokine producing cells were determined using Boolean gating.

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HLA-DR and HLA-ABC expression. PBMCs from DRB1*15:01^{+} or DRB1*15:01^{-} patients with Parkinson's disease ($n=5$ for both) and healthy controls ($n=3$ DRB1*15:01^{+} and $n=5$ DRB1*15:01^{-}) were assessed for HLA-DR and HLA-ABC (as a control) expression. 721.221 and RM3 cells (both sourced from ATCC, mycoplasma free) were used as controls for HLA-DR and HLA-ABC expression. 721.221 cells lack HLA-ABC and express HLA-DR, whereas RM3 cells lack HLA-DR and express HLA-ABC. All cells were stained for cell-surface antigens CD14 (anti-CD14–APC, 61D3, Tonbo biosciences), CD3 (anti-CD3–AF700, UCHT1, BD Pharmingen), HLA-ABC (anti-HLA-ABC–AF488, W6/32; pan-HLA class I, BioLegend), HLA-DR (anti-HLA-DR–PE, L243; pan-HLA-DR, eBioscience), and fixable viability dye eFluor 506 (eBioscience) or isotype controls for HLA-ABC (AF488 mouse IgG2a, κ, catalogue number 400233, BioLegend) or HLA-DR (PE mouse IgG2a, κ, catalogue number 12-4724, eBioscience). After washing, cells were fixed using 4% paraformaldehyde. Samples were acquired on a BD LSR II flow cytometer. The fraction of living cells expressing HLA-ABC or HLA-DR was determined using FlowJo X Software.

α-Syn purification and α-syn PFF preparation. The recombinant α-syn monomer was purified as previously described[44]. α-Syn pre-formed fibrils (PFF) were prepared by agitating α-syn monomer in a transparent glass vial with a magnetic stirrer (350 r.p.m. at 37 °C). After 5–7 days of agitation, the clear α-syn monomer solution became turbid, indicative that α-syn fibrils were generated. The α-syn fibrils were then sonicated for 30 s at 10% amplitude to generate α-syn PFF (Branson Digital Sonifier). α-Syn monomer and PFF were aliquoted and kept at −80 °C.

Statistics and reproducibility. A power analysis was not conducted a priori as there was no means to estimate effect size. Future validation studies will test whether the Y39 antigenic region is recognized significantly higher in patients with Parkinson's disease compared to healthy controls. The recognition frequency of this peptide was 17% in patients with Parkinson's disease and 3% in healthy controls, which achieves 61% power to detect a response difference between response rates of 14 percentage points. To achieve 80% power in a repeat study to detect a similar effect size, a total of 62 patients with Parkinson's disease and 62 healthy controls should be included. Additionally validation studies will test whether the overall recognition of the 11 peptides is significantly higher in patients with Parkinson's disease compared to healthy controls. On the basis of our combined cohort data, the recognition frequency of a pool of peptides was 37% in patients with Parkinson's disease and 8% in healthy controls. To obtain 80% power in a validation study a cohort size of 43 in both patients with Parkinson's disease and healthy controls will be required to detect the same effect.

The Fisher's exact (two-tailed) test was used to evaluate the contingency between carriers and non-carriers of the DRB1*15:01 and DRB5*01:01 alleles in the patients with Parkinson's disease and healthy control donors (Supplementary Table 3), between the responses to phosphorylated S129 epitopes of patients with Parkinson's disease and healthy control donors (Fig. 1e–g), and between DRB1*01/DRB5*01:01/DQB1*03:04/A*11:01 carriers and non-carriers in patients with Parkinson's disease and healthy controls (Table 1). A non-parametric test was used because the data are not normally distributed. A Fisher's exact test that provides exact P values for the analysis of contingency tables is available in most professional statistical analysis packages.

The Mann–Whitney test (two-tailed) was used to assess whether the number of SFC of healthy control donors would be less or greater than those of donors with Parkinson's disease (Figs 1b–g, 2a–c and Extended Data Fig. 1). The Mann–Whitney U-test (two-tailed) was used to determine whether the number of IFNγ SFC was different from the number of IL-5 SFC in patients with Parkinson's disease (Fig. 2d). A non-parametric test was used because the data are not normally distributed.

Student's t-tests were used to analyse parametric differences in demographics between patients with Parkinson's disease and healthy control donors (Supplementary Tables 1a, b, 2).

The Wilcoxon signed-rank test was used to analyse differences in population means of the repeated measurements of number of SFC induced by medium and different isoforms of α-syn (Extended Data Fig. 3). A non-parametric test was used because the data are not normally distributed. We hypothesized that responses to proteins and peptides would be higher than medium alone, therefore a one-tailed test was used for those comparisons. Comparison between PFF and native α-syn was two-tailed.

Data availability. All data generated or analysed during this study are included in this article and its Supplementary Information.

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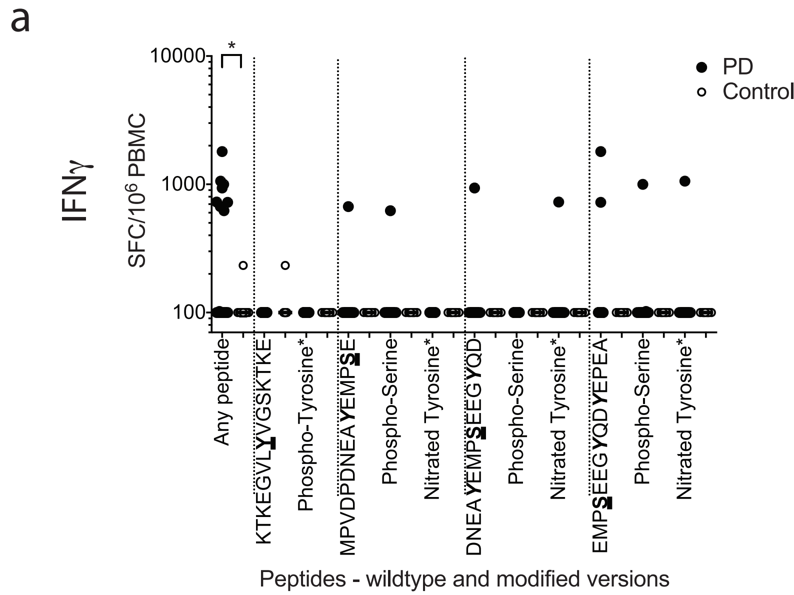

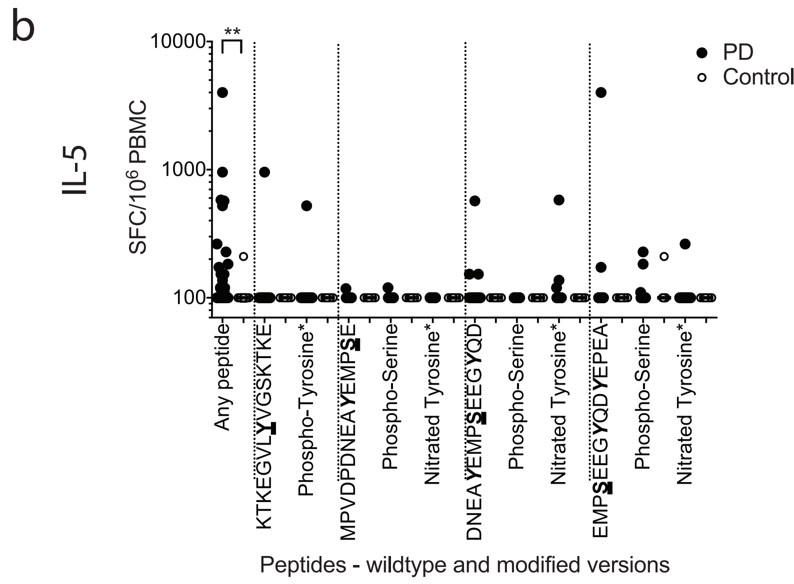

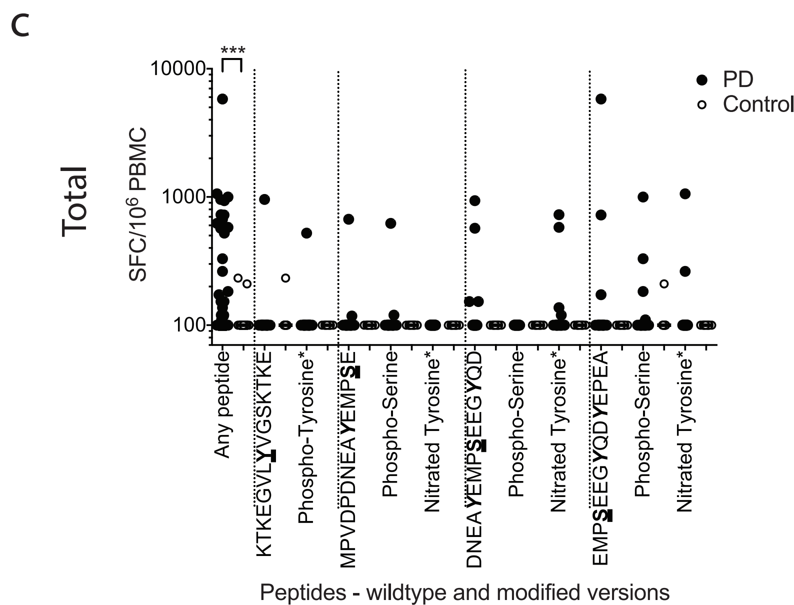

Extended Data Figure 1 | T cell reactivity against (wild-type and post-translationally modified) α-syn peptides. Magnitude of responses, expressed as the total magnitude (SFC per 10^6 PBMCs) of response per peptide and participant combination. Responses against any α-syn 15-mer peptide spanning S129 and Y39, 'any peptide', Parkinson's disease ($n = 209$), control ($n = 132$); and responses against individual α-syn 15-mer peptides spanning S129 and Y39. Each dot represents a peptide and participant combination. Closed circles, Parkinson's disease ($n = 19$); open circles, control ($n = 12$). Two-tailed Mann–Whitney U-test, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. **a**, IFNγ response. **b**, IL-5 response. **c**, Total (IFNγ and IL-5 combined) response.

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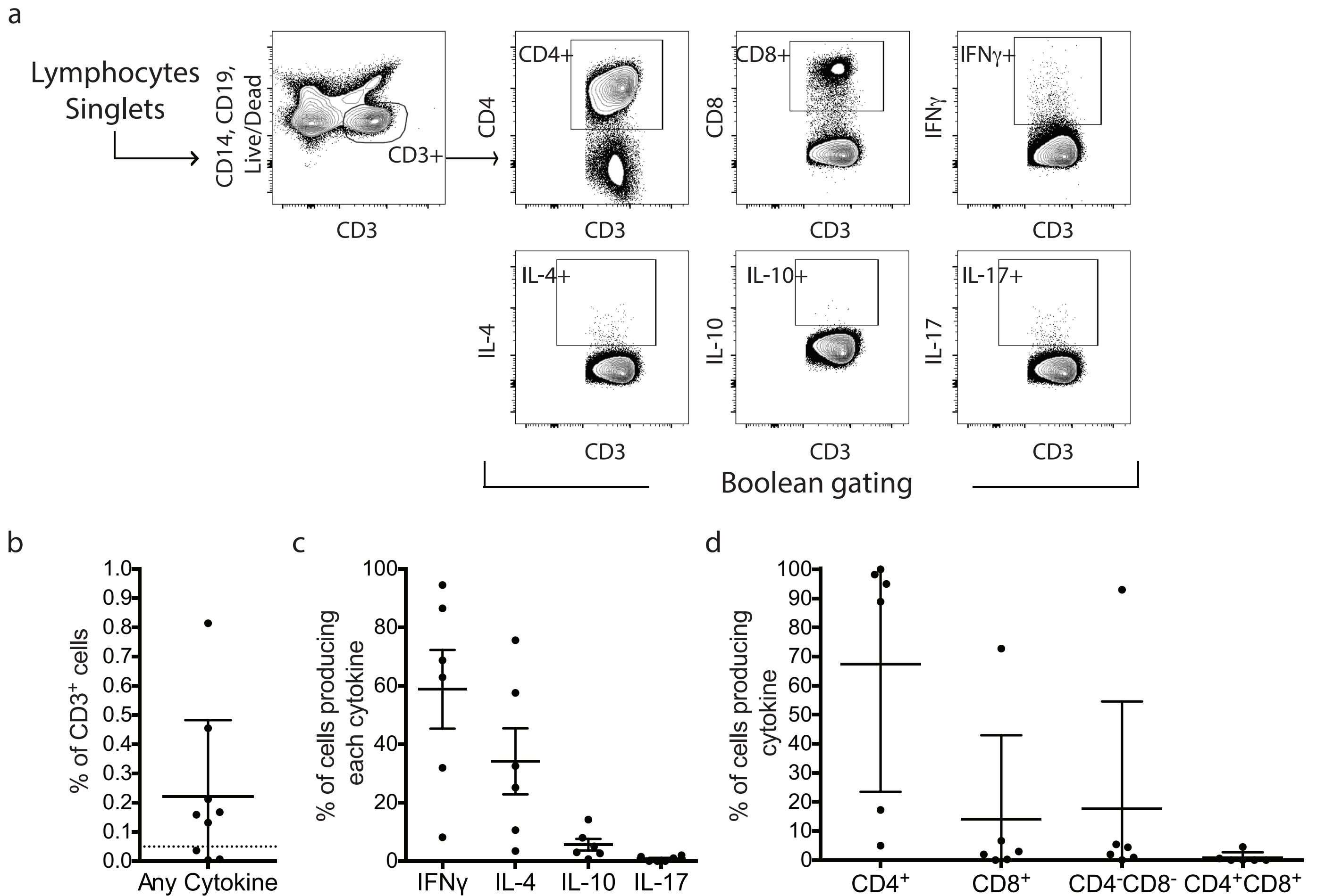

Extended Data Figure 2 | Characterization of α-syn-specific responses in Parkinson's disease. a, Gating strategy. T cells were gated based on CD3 expression. Boolean gating was used to define cytokine-producing cells expressing CD4 and/or CD8. **b**, Percentage of total cytokine detected from $CD3^+$ T cells in response to α-syn peptides. Each point represents one participant ($n = 9$); mean ± s.d. are indicated. Dotted line indicates 0.05% cut-off for specific cytokine production by $CD3^+$ T cells. **c**, Percentage of responding T cells that produce each cytokine, IFNγ, IL-4, IL-10 and IL-17. Each point represents one participant that exceeded the cut-off ($n = 6$), mean ± s.d. are indicated. **d**, Percentage of responding T cells that are $CD4^+$, $CD8^+$, $CD4^-CD8^-$, or $CD4^+CD8^+$ T cells. Each point represents one participant ($n = 6$), mean ± s.d. are indicated.

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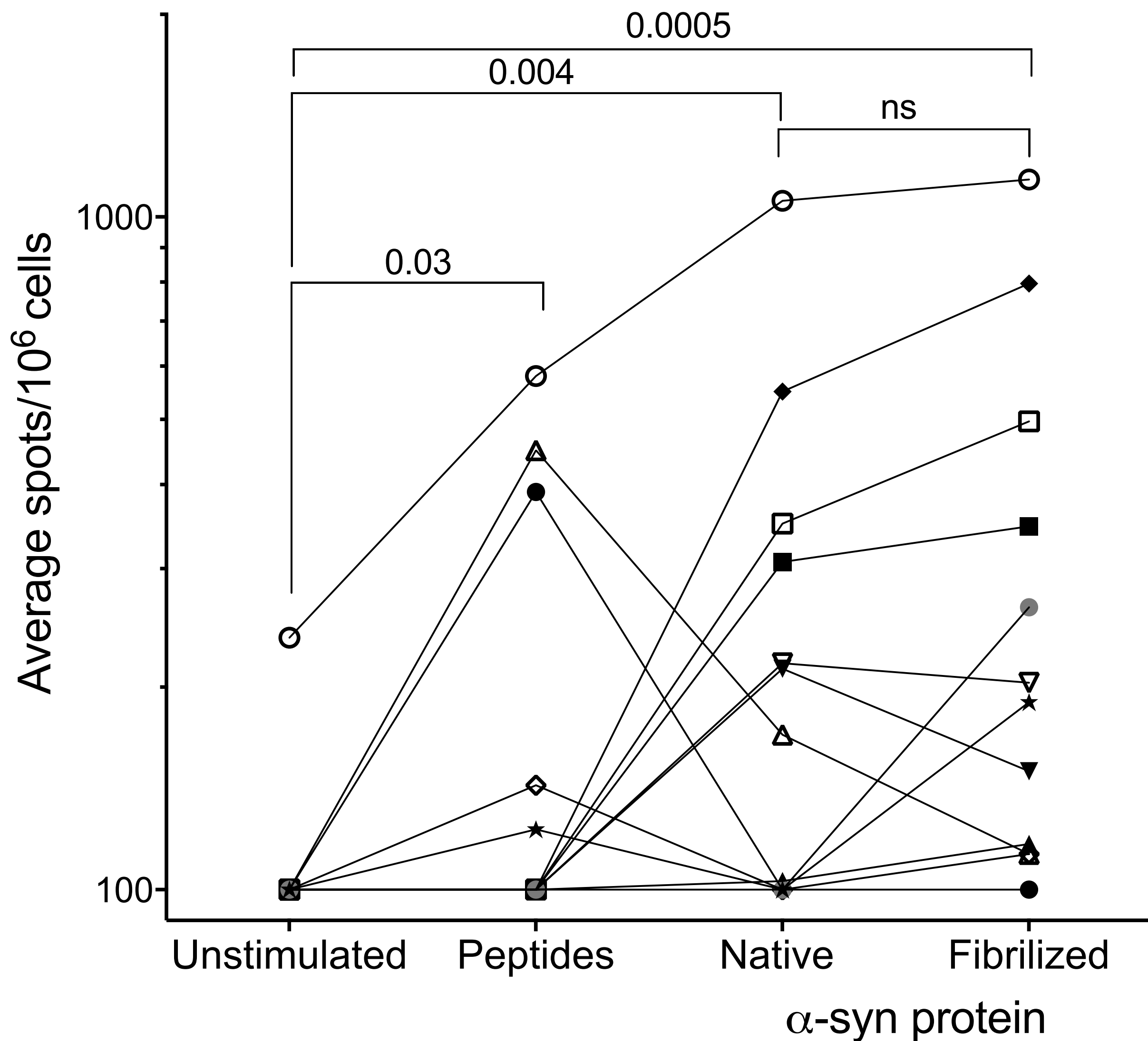

Extended Data Figure 3 | Specific T cell reactivity against native or fibrilized α-syn. Magnitude of responses, expressed as the average spots per 10^6 PBMC, of response per protein and participant with Parkinson's disease or peptide and participant with Parkinson's disease combination ($n = 12$ Parkinson's disease participants, each represented by a different symbol). The lines connect discrete values from each individual participant and are present to provide a means to compare responses within and between individuals. The difference between response to unstimulated compared to peptides, the native α-syn and PFF groups is significant by the Wilcoxon signed-rank one-tailed test (values are shown in the figure). No significant difference (Wilcoxon signed-rank two-tailed test) in response to PFF and native protein was apparent in this relatively small sample.

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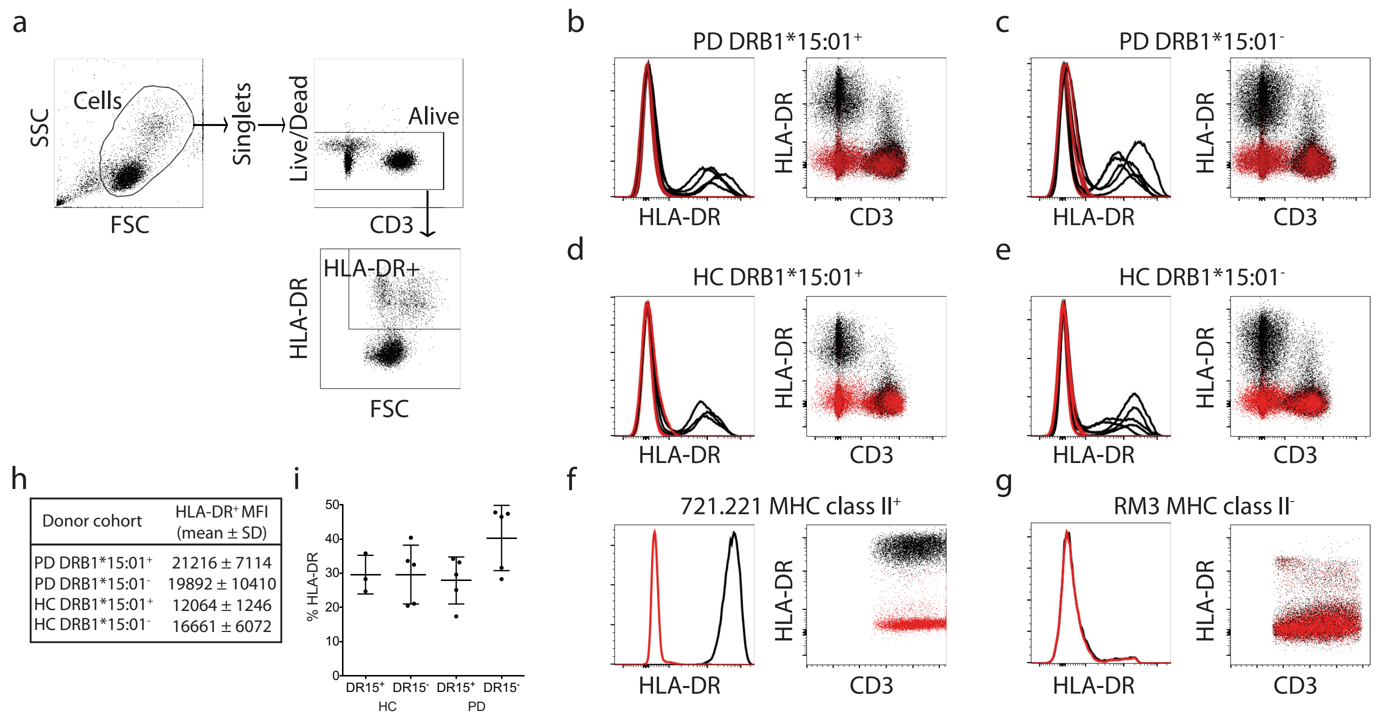

Donor cohort	HLA-DR^{+} MFI (mean ± SD)
PD DRB1*15:01^{+}	21216 ± 7114
PD DRB1*15:01^{-}	19892 ± 10410
HC DRB1*15:01^{+}	12064 ± 1246
HC DRB1*15:01^{-}	16661 ± 6072

Extended Data Figure 4 | HLA-DR surface expression across DRB1*15:01^{+} or DRB1*15:01^{-} participants with Parkinson's disease and healthy controls. a, Gating strategy for FACS analysis. After eliminating non-lymphocytes and doublet cells by forward- and side-scatter, cells were gated based on HLA-DR expression. **b–e**, HLA-DR and CD3 expression of cells from participants (black, HLA-DR antibody; red, isotype control) with Parkinson's disease that carry (**b**; $n = 5$) and do not carry (**c**; $n = 5$) the DRB1*15:01 allele and healthy controls (HC) that carry (**d**; $n = 3$) and do not carry (**e**; $n = 5$) the DRB1*15:01 allele. **f**, **g**, 721.221 (**f**) and RM3 (**g**) cells are used as controls that do not and do express HLA class II, respectively. **h**, Mean fluorescent intensities (MFI) ± s.d. of HLA-DR expression for each participant cohort. **i**, Percentage of living cells that express HLA-DR. Data are mean ± s.d.

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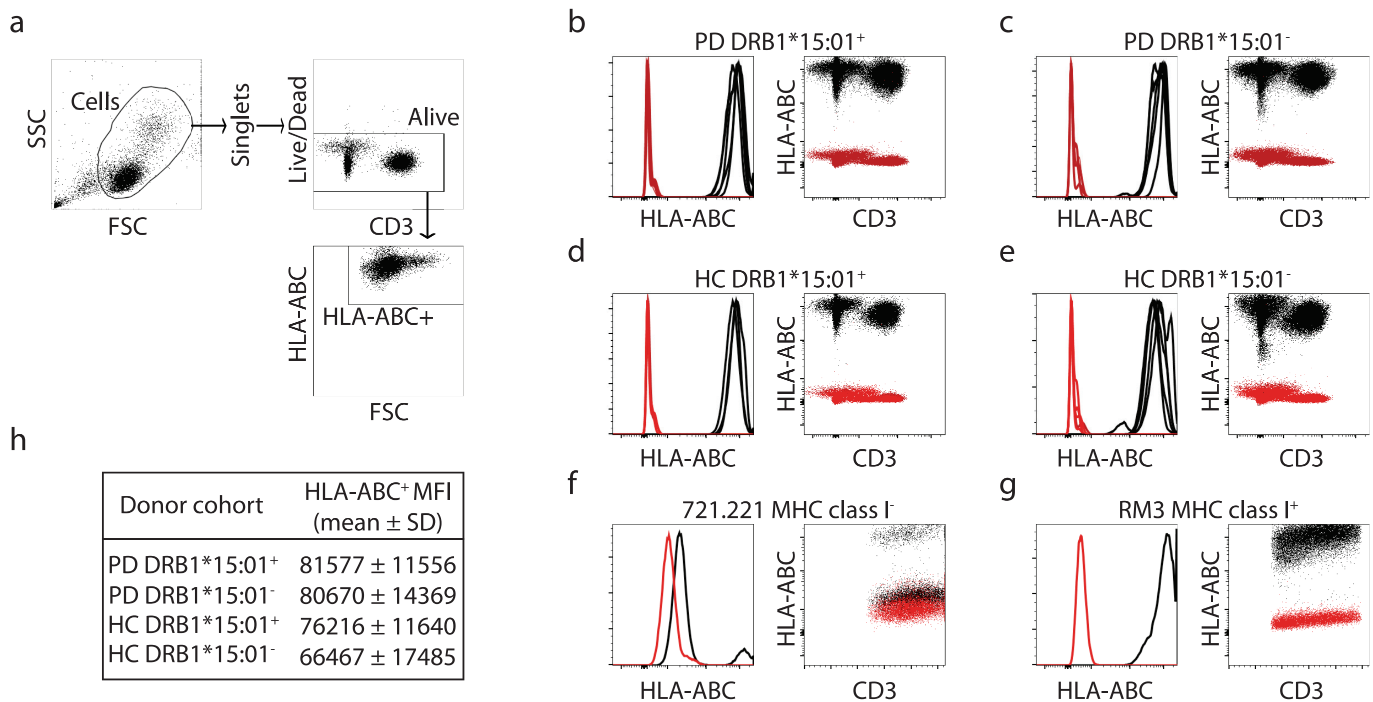

Donor cohort	HLA-ABC+ MFI (mean ± SD)
PD DRB1*15:01+	81577 ± 11556
PD DRB1*15:01-	80670 ± 14369
HC DRB1*15:01+	76216 ± 11640
HC DRB1*15:01-	66467 ± 17485

Extended Data Figure 5 | HLA class I surface expression across DRB1*15:01^{+} or DRB1*15:01^{-} participants with Parkinson's disease and healthy controls. a, Gating strategy for FACS analysis. After eliminating non-lymphocytes and doublet cells by forward- and side-scatter, cells were gated based on HLA-ABC expression. **b**–**e**, HLA-ABC and CD3 expression of cells from participants (black, HLA-ABC antibody; red, isotype control) with Parkinson's disease that carry (**b**; $n = 5$) and do not carry (**c**; $n = 5$) the DRB1*15:01 allele and healthy controls that carry (**d**; $n = 3$) and do not carry (**e**; $n = 5$) the DRB1*15:01 allele. **f**, **g**, 721.221 (**f**) and RM3 (**g**) cells are used as controls that do not and do express HLA class I, respectively. (**h**) Mean fluorescent intensities (MFI) ± s.d. of HLA-ABC expression for each participant cohort.

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